

What is claimed is:

1. A method for enhancing the detection of a polynucleotide separated by

Matched Ion Polynucleotide Chromatography comprising:

a) covalently attaching a chemical tag to said polynucleotide to form a

5 tagged polynucleotide,

b) applying said tagged polynucleotide to a separation medium having

a non-polar surface,

c) eluting said tagged polynucleotide from said surface with a mobile

phase containing a counterion agent and an organic solvent,

10 and

d) detecting said tagged polynucleotide, wherein said medium is

characterized by having a DNA Separation Factor of at least

0.05.

2. A method of Claim 1 wherein said tag comprises a fluorescent group.

15 3. A method of Claim 2 wherein said fluorescent group is selected from the

group consisting of 5-carboxyfluorescein, 2',7'-dimethoxy-4',5'-

dichloro-6-carboxyfluorescein, N,N,N',N'-tetramethyl-6-

carboxyrhodamine, 6-carboxy-X-rhodamine, Fluorescein, Rhodamine,

BODIPY-TR-X, and Cascade Blue, and Alexa 350.

20 4. A method of Claim 1 wherein said tag absorbs at a wavelength different

from said polynucleotide.

5. A method of Claim 4 wherein said tag is selected from the group consisting of porphyrin derivative, 5-carboxyfluorescein, 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein, N,N,N',N'-tetramethyl-6-carboxyrhodamine, 6-carboxy-X-rhodamine, Fluorescein, Rhodamine, BODIPY-TR-X, Cascade Blue, and Alexa 350.
6. A method of claim 1 wherein said separation medium is characterized by having a Mutation Separation Factor of at least 0.1.
7. A method of claim 1 wherein said separation medium is substantially free from contamination with multivalent cations.
8. A method of claim 1 wherein said medium comprises polymer beads having an average diameter of 0.5 to 100 microns and having a surface composition essentially completely substituted with a moiety selected from the group consisting of unsubstituted, methyl, ethyl, hydrocarbon, and hydrocarbon polymer, wherein said hydrocarbon optionally has from 23 to 1,000,000 carbons, wherein said hydrocarbon polymer optionally has from 23 to 1,000,000 carbons.
9. A method of claim 1 wherein said medium comprises beads having an average diameter of 0.5 to 100 microns, the beads comprising nonporous particles coated with a hydrocarbon or non-polar hydrocarbon substituted polymer, or particles having substantially all polar groups reacted with a non-polar hydrocarbon or substituted hydrocarbon group, wherein said particles are a member selected from the group consisting of silica, silica carbide, silica nitrite, titanium

oxide, aluminum oxide, zirconium oxide, carbon, insoluble polysaccharide, and diatomaceous earth.

10. A method of claim 1 wherein said medium comprises a polymeric monolith.

5 11. A method of claim 1 wherein said medium comprises a derivatized silica gel monolith.

12. A method of claim 1 wherein said tagged polynucleotide comprises a PCR amplification product obtained by providing a PCR primer having a covalently bound tag during a PCR amplification wherein said tag is
10 incorporated into said PCR amplification product.

13. A method for enhancing the detection of a polynucleotide separated by Matched Ion Polynucleotide Chromatography comprising:

- a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
- 15 b) applying said tagged polynucleotide to a separation bed of Matched Ion Polynucleotide Chromatography particles,
- c) eluting said tagged polynucleotide from said particles with a mobile phase containing a counterion agent and an organic solvent, and
- 20 d) detecting said tagged polynucleotide, wherein steps (b) and (c) are performed in a system for separating a mixture of polynucleotide fragments comprising a chromatographic

5 column having two ends, said column containing said separation bed of Matched Ion Polynucleotide Chromatography separation particles held in the column between porous frits positioned at each end thereof, said column having an inlet, an injection valve in communication with said inlet through a flow path therebetween, mobile phase supply means in communication with said injection valve through at least one flow path therebetween, and multivalent cation capture resin, selected from cation exchange resin and chelating resin, positioned in said flow path, said multivalent cation capture resin being capable of removing multivalent cations from aqueous solutions, whereby any multivalent cation contaminants in said flow path are removed before said contaminants contact the separation bed.

- 15 14. A method for enhancing the detection of a polynucleotide separated by Matched Ion Polynucleotide Chromatography comprising:
- 20 a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,
- b) applying said tagged polynucleotide to a separation bed of Matched Ion Polynucleotide Chromatography particles,
- c) eluting said tagged polynucleotide from said particles with a mobile phase containing a counterion agent and an organic solvent,

d) detecting said tagged polynucleotide, wherein steps (b) and (c) are performed in a system for separating a mixture of polynucleotide fragments the system comprising a chromatographic column having two ends, said column containing a separation bed of Matched Ion Polynucleotide Chromatography separation particles held in the column between porous frits positioned at each end thereof, said column having an inlet, an injection valve in communication with said inlet through a conduit, eluant supply means in communication with said injection valve through at least one conduit, wherein said porous frits, chromatographic column, injection valve, eluant supply means, and conduits have process solution-contacting surfaces which contact process solutions held therein or flowing therethrough, and wherein the process solution-contacting surfaces of said porous frits are material which does not release multivalent cations into aqueous solutions flowing therethrough.

15. A method for increasing the retention time of a polynucleotide separated by Matched Ion Polynucleotide Chromatography comprising:

a) covalently attaching a chemical tag to said polynucleotide to form a tagged polynucleotide,

b) applying said tagged polynucleotide to a separation medium having a non-polar surface,

c) eluting said tagged polynucleotide from said surface with a mobile phase containing a counterion agent and an organic solvent,

d) detecting said tagged polynucleotide, wherein said medium is characterized by having a DNA Separation Factor of at least 0.05, wherein said chemical tag is non-polar.

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16. A method of Claim 15 wherein said tag comprises a hydrocarbon group, wherein said hydrocarbon group is selected from the group consisting of alkyl, cycloalkyl, aryl and arylalkyl groups.

17. A method for enhancing the detection of a polynucleotide separated by Matched Ion Polynucleotide Chromatography, comprising:

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a) contacting said polynucleotide with a reversible DNA-binding dye to form a complex between said polynucleotide and said reversible DNA-binding dye,

b) applying said complex to a separation medium having a non-polar surface,

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c) eluting said complex from said surface with a mobile phase containing a counterion agent and an organic solvent, and

d) detecting said complex.

18. A method of claim 17 in which said reversible DNA-binding dye is selected from the group consisting of DNA intercalator dye and DNA groove binding dye.

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19. A method of claim 17 in which said reversible DNA-binding dye is selected from the group consisting of PICO GREEN, ethidium bromide, propidium iodide, Acridine orange, 7-aminoactinomycin D, cyanine dye, Bisbenzimidazole, Benzoxanthene yellow, Netropsin, Indole dye, Imidazole dye, and Actinomycin D.
20. A method for the detection of a mutation in a sample double stranded DNA fragment comprising:
- a) covalently attaching a chemical tag to at least one of said sample DNA fragment and a corresponding wild type fragment to form a tagged polynucleotide,
 - b) hybridizing said sample DNA fragment with said corresponding wild type DNA fragment to form a mixture of homoduplexes and heteroduplexes if a mutation is present in said sample DNA fragment,
 - c) applying the product of step (b) to a separation medium having a non-polar separation surface, and
 - d) eluting said mixture with a mobile phase containing a counterion agent and an organic solvent where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where said eluting results in the separation of said heteroduplexes from said homoduplexes.

21. A method of claim 20 in which a different uniquely detectable tag is covalently attached to each strand of said sample DNA.
22. A method of claim 20 in which a different uniquely detectable chemical tag is covalently attached to each strand of said wild type fragment.
- 5 23. A method of claim 20 in which said wild type fragment in step (b) is tagged and the amount of said wild type fragment is added in excess of said sample DNA.
24. A method for increasing the melting temperature of a double stranded DNA as determined by temperature titration using by Matched Ion Chromatography, comprising covalently binding a non-polar chemical tag to said DNA, to form a tagged polynucleotide, prior to said separation.
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25. A method of claim 24 wherein said non-polar chemical tag comprises a hydrocarbon group, wherein said hydrocarbon group is selected from the group consisting of alkyl, cycloalkyl, aryl and arylalkyl groups.
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26. A method of claim 24 wherein said non-polar tag comprises a fluorescent group.
27. A method of claim 24 wherein said non-polar tag is bound at an end of said DNA.

28. A method for detecting a covalently tagged polynucleotide separated by
Matched Ion Polynucleotide Chromatography comprising:

a) applying said tagged polynucleotide to a separation medium having
a non-polar surface,

b) eluting said tagged polynucleotide from said surface with a mobile
phase containing a counterion agent and an organic solvent,
and

c) detecting said tagged polynucleotide, wherein said medium is
characterized by having a DNA Separation Factor of at least
0.05.

29. A method of Claim 28 wherein said tag comprises a fluorescent group.

30. A method of Claim 28 wherein said tag absorbs at a wavelength different
from said polynucleotide.

31. A method of claim 28 wherein said separation medium is characterized
by having a Mutation Separation Factor of at least 0.1.

32. A method of claim 28 wherein said separation medium is substantially
free from contamination with multivalent cations.

33. A method for detecting a complex comprising a polynucleotide bound to a
reversible DNA-binding dye, as separated by Matched Ion
Polynucleotide Chromatography, comprising:

- a) applying said complex to a separation medium having a non-polar surface,
- b) eluting said complex from said surface with a mobile phase containing a counterion agent and an organic solvent, and
- 5 c) detecting said complex, wherein said medium is characterized by having a DNA Separation Factor of at least 0.05.